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Patent Office

(21) (A1) 2,101,079
(22) 1993/07/21
(43) 1994/08/09

99/5,680,5

(51) INTL.CL.⁵ C08B-037/08

(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Process for Preparing Biomedical Grade Chitin and Chitosan

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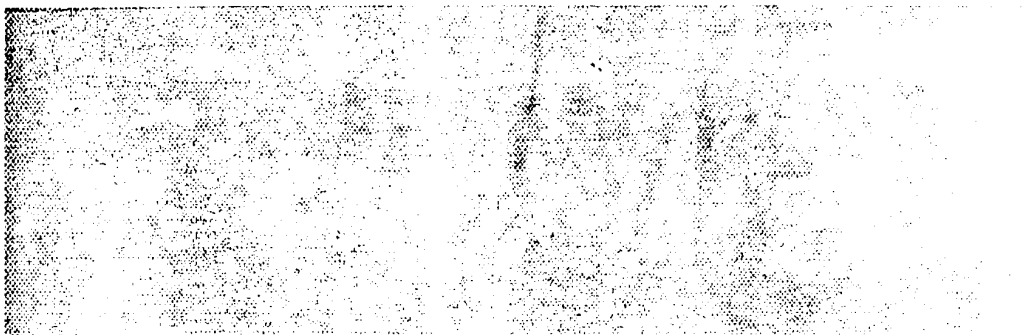
(30) (KR) 93-1687 1993/02/08

(57) 14 Claims

Notice: This application is as filed and may therefore contain an incomplete specification.

Canada

CCA 3254 (10-92) 41 7530-21-938-3254



PROCESS FOR PREPARING BIOMEDICAL GRADE
CHITIN AND CHITOSAN

Field of the Invention

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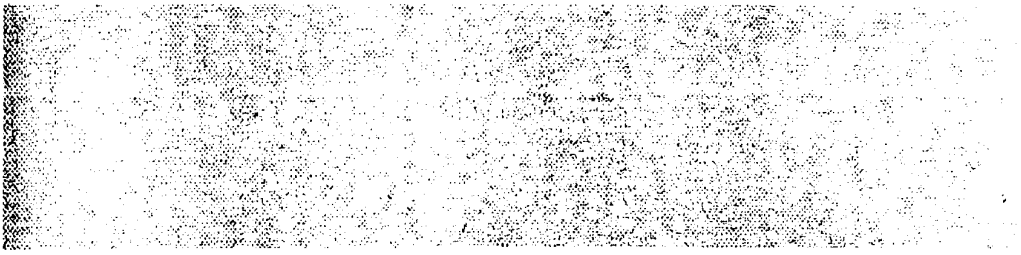
The present invention relates to a process for the preparation of chitin and chitosan, and, particularly, to a process for preparing a biomedical grade chitin of a high purity and whiteness with a high molecular weight and a biomedical grade chitosan of a high molecular weight with a
10 near-complete level of deacetylation.

Background of the Invention

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As a second most abundantly available polysaccharide, chitin is a high value-added biopolymer normally extracted from the shells of crustaceans (e.g., shrimp and crab). However, since chitin is water-insoluble, it is conventionally converted to chitosan--which is water soluble, of high
20 molecular weight linear polyamines and industrially more useful than chitin--by a deacetylation reaction.

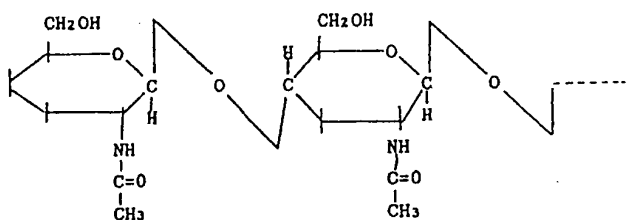
With the abundance of shellfish wastes available, many commercial studies have been made on various applications of the chitin extracted from such shells. During the early days,
25 chitin and chitosan derivatives were mainly employed as a coagulant useful for recovering valuable materials, e.g.,



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protein from the food wastes. Recently, their applications have been expanded further into such various fields as food industry, medicines, biochemistry involving the use of a membrane, enzyme and microorganism-immobilizing carrier, cosmetics, agriculture, chemical engineering, environmental protection and the like.

Chitin is conventionally prepared from shells by a method comprising pretreatment, deproteinization and demineralization thereof; and, the end product is characterized by having a structure of the following formula:

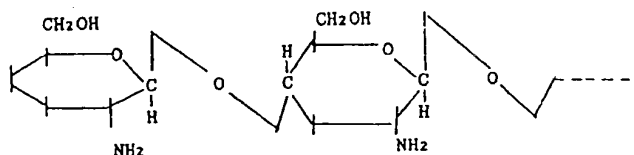


One of the well-known methods for preparing chitin is the one proposed by Whistler & BeMiller, which comprises: washing and drying crab shells in a vacuum oven at 50°C; grinding and soaking the dried shells in 10% NaOH solution at a room temperature for 3 days to deproteinize them; washing the deproteinized material with water until freed of alkali and then with an organic solvent and drying the resulting white material under a reduced pressure; introducing the dried material into 37% HCl solution at -20°C for 4 hours to produce a crude chitin; washing the crude chitin with cold water and

with the organic solvent; and repeating said swelling in a cold HCl solution and washing procedures to produce chitin as a final product.

Alternatively, chitin may be prepared by another well-known Hackmann method, which comprises: washing and drying shells in an oven at 100°C; digesting the dried shells with 2N HCl at a room temperature for 5 hours; washing, drying and grinding the digested materials; extracting finely ground materials with 2N HCl at 0°C for 2 days under stirring occasionally; washing the extracted materials with water; extracting the washed materials with 1N NaOH at 100°C for 12 hours under occasional stirring; and repeating the NaOH treatment four times or more.

In contrast, chitosan is prepared by deacetylation of chitin by a biological or chemical treatment; and, characterized by the structure of the following formula:



As shown in the above formula, since chitosan has free amino groups, it tends to be chemically reactive.

Specifically, chitosan can be easily obtained by deacetylizing the chitin obtained in accordance with a known

method as described above with a 30-50% NaOH solution for 5-20 hours.

5 However, since the prior art processes for preparing chitin are practised under relatively severe reaction conditions for the purpose of reducing the protein or CaCO_3 content therein, there are several disadvantages: that is, in the above Whistler & BeMiller method, since the deproteinization with NaOH solution is followed by the demineralization with conc. HCl solution, breakage in the
10 chain of chitin may occur, thereby resulting in a chitin having a lower molecular weight; the chitin so obtained is liable to discolor when exposed to air, has a relatively higher content of impurities and, therefore, may not be suitable for such applications as biomedical fields and food
15 industries; and, further, even though mineralization conducted at very low temperature the use of concentrated HCl solution may also cause breakage in the chain. On the other hand, in Hackmann method, the ambient or higher temperature used during the HCl treatment for removing CaCO_3 may cause breakage in the
20 main chain of chitin.

To solve the above-mentioned problems, therefore, the present inventors have carried out extensive research to discover a novel process capable of producing biomedical grades of chitin and chitosan by way of carefully controlling
25 the reaction conditions, especially the temperature employed during the demineralization step and the use of an inert gas

during the deproteinization step.

Summary of the Invention

5 Accordingly, it is an object of the present invention to provide a process for preparing a biomedical grade chitin having a high purity, high molecular weight and enhanced whiteness.

10 Another object of the present invention is to provide a process for preparing a biomedical grade chitosan with a high molecular weight and almost complete of deacetylation, from the biomedical grade chitin prepared in accordance with the present invention.

15 As one aspect of the present invention, there is provided a process for preparing a biomedical grade chitin, which comprises the steps of:

(A) drying and crushing crustaceous shells to provide a finely crushed shell powder;

20 (B) digesting initially the powder in a HCl solution at a temperature ranging from -10 to 10°C for a period ranging from 10 to 25 hours, and, thereafter, further digesting the powder in the HCl solution at a temperature ranging from 10 to 20°C for a period ranging from 2 to 8 hours;

(C) washing with water, filtering and rinsing with a solvent said HCl-treated material to provide a crude chitin;

25 (D) soaking and heating the crude chitin in a NaOH

solution; and

(E) washing with water, filtering and rinsing with a solvent said NaOH-treated chitin to produce the biomedical grade chitin.

5 Another aspect of the invention resides in a process for preparing a biomedical grade chitosan, which comprises the steps of:

(a) soaking the chitin prepared in accordance with the method of the present invention in a NaOH solution at a temperature ranging from 80 to 100°C for a period ranging from 2 to 12 hours to provide a crude chitosan;

(b) filtering, washing with water, soaking in deionized water and then in an aqueous mixture of a water-miscible organic solvent, filtering and drying the crude chitosan; and

15 (c) repeating the above-mentioned steps (a) and (b) by using the crude chitosan once or more to provide said biomedical grade chitosan.

Detailed Description of the Invention

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Specifically, the process for preparing a biomedical grade chitin in accordance with the present invention comprises the following three stages of operation.

25

Pretreatment

Crustaceous shells may be preferably pretreated in

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various manners. First of all, the shells may be normally treated with hot water, e.g., soaking in hot water at a temperature ranging from 40 to 50°C for a period of 30 minutes to 1 hour so as to remove the remnants and impurities from the shells.

5 Subsequently, the shells are washed with water, dried by means of a dehydrator and soaked in an organic solvent at a room temperature for a period of 30 minutes to 1 hour. Examples of the organic solvent which may be used include: 10 ethanol, methanol, acetone, tetrahydrofuran (THF), dioxane and methyl ethyl ketone (MEK). The shells are then air dried in the shade to a water content of 8-12%. If the dried shells are not to be employed immediately, it is important to 15 maintain the shells at a dried and cooled state before further use. If maintenance of the shells is not good, it may decay the chitin obtained therefrom to lower the molecular weight thereof.

The dried shells are then preferably crushed to fine powdery particles having a mean particle size of 0.5 to 3mm or 20 of 200 to 300 meshes by using a conventional mill.

Demineralization

The crushed shells (having water contents of 8-12%) are then subjected to a HCl treatment so as to remove any mineral components, including CaCO_3 , thereby to provide a mineral-free 25 chitin.

The HCl treatment can be conducted in two steps by digesting initially the shell powder, pretreated as described previously, in an aqueous HCl solution at a temperature ranging from -10 to 10°C, preferably -5 to 5°C for a period ranging from 10 to 25 hours, preferably 15 to 20 hours, while stirring by means of a mechanical stirrer; and, thereafter, further digesting in said HCl solution at a temperature ranging from 10 to 20°C, preferably 12 to 18°C, for a period ranging from 2 to 8 hours, preferably 3 to 5 hours. It should be noted that the temperature in the further digestion is raised rapidly from the initial temperature. The HCl aqueous solution can be employed in a concentration ranging from 0.3 to 4M. The mechanical stirrer can be employed at a stirring speed ranging from 50 to 200 rpm, preferably at 100 rpm; and, if the speed is greater than 200 rpm, the efficiency of the HCl treatment becomes lower. During the HCl treatment, the used HCl solution is preferably replaced with a fresh HCl solution once or twice.

Alternatively, the HCl treatment may be conducted in one step by digesting the dried shell powder in an aqueous HCl solution at a temperature ranging from -10 to 8°C, preferably -5 to 5°C, for a period ranging from 20 to 60 hours, preferably 30 to 50 hours while stirring.

The material obtained by filtration after the HCl treatment is washed with water to remove any remaining acidic components, or adjusted to a pH of 7-9 by the addition of a

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NaOH solution, and then soaked in a deionized water for a given period, e.g., 1 hour to 6 hours.

5 The material obtained after filtration is then rinsed once or twice with an organic solvent and dried to provide a crude chitin. Examples of the organic solvent which may be used include: ethanol, methanol, acetone, THF, dioxane, MEK and the like. The rinsing process with the solvent further brings about an improvement in the whiteness of the chitin obtained and, therefore, no additional use of an oxidizing or
10 reducing agent as decolorant is required in the present invention.

Deproteinization

15 The crude chitin thus obtained is then subjected to deproteinization and washing procedures, thereby to provide a biomedical grade chitin, as follows.

The deproteinization process can be carried out by soaking and heating the crude chitin in an aqueous NaOH solution while introducing an inert gas continuously. The
20 aqueous NaOH solution is suitably employed in a concentration ranging from 2 to 10% by weight. The NaOH treatment is generally carried out at a temperature ranging from 80 to 100°C for a period ranging from 2 to 4 hours. Examples of the
25 useable inert gas include: nitrogen, argon, helium and neon gases; and the introduction of the inert gas results in preventing the breakage of the chitin chain.

Subsequently, the material obtained by filtration after the NaOH treatment is washed with water to remove any remaining NaOH component, or adjusted to pH of 7-8 by the addition of a HCl solution and then soaked in deionized water for a given period, e.g., 1 hour to 5 hours. The material obtained after filtration is then rinsed once or twice with said organic solvent described above and dried to produce a biomedical grade chitin possessed with a high level of purity, molecular weight and whiteness.

If desired, the above-mentioned NaOH treatment and rinsing procedures may be repeated twice or more; and, further, the chitin obtained may be soaked in deionized water for 2 to 5 days and then filtered and dried under a vacuum to further enhance the whiteness thereof.

In accordance with another aspect of the present invention, chitosan possessed with a higher molecular weight and deacetylation degree can be obtained from the chitin prepared by the method of the present invention, as follows.

First, the chitin prepared as described above is treated with a NaOH solution to provide a crude chitosan. The NaOH treatment can be carried out by adding a 30 to 50 wt% NaOH aqueous solution to the chitin and heating the mixture at a temperature ranging from 80 to 100°C for a period ranging from 2 to 12 hours while introducing an inert gas continuously. The inert gas used may be any one of those described above.

Subsequently, the crude chitosan is washed and soaked in

deionized water, and then in an aqueous mixture of a water miscible organic solvent, filtered, and dried to provide a relatively pure chitosan. The soaking process serves to improve the whiteness of the chitosan. Examples of the organic solvent which may be used in the soaking process include: acetone, ethanol, methanol, isopropanol, dioxane, THF, MEK and the like; and, the mixture may comprise a 5 to 30% by weight of the solvent.

Finally, the above-mentioned NaOH treatment and purifying procedures are repeated by using the chitosan once to provide a biomedical grade chitosan possessed with a high degree of deacetylation.

If desired, in order to obtain such pure chitosan, the above-mentioned NaOH treatment and purifying processes may be further repeated.

The following Examples are intended to further illustrate the present invention more specifically, without limiting the scope of the invention.

In the Examples, the content of remaining CaCO_3 in chitin was determined by measuring the residues on ignition after the combustion thereof in a furnace at 800°C for 1 hour. The protein content was determined by using a protein assay kit, a product of Bio-Rad, U.S.A., and a UV/VIS spectrophotometer, a product of Hewlett-Packard, U.S.A., in accordance with the Bradford method. Further, the mineral content was determined by using ICP-AES (Inductively Coupled Plasma Atomic Emission

Spectrophotometer), a product of Seiko, Japan, and an AA(Atomic Absorption) Spectrophotometer, a product of Perkin Elmer, U.S.A. The degree of deacetylation was evaluated in accordance with the Mima method described in J. Appl. Polym. Sci. 28, 1909(1983) using an IR(Infrared) Spectrophotometer. The whiteness property was evaluated by standing the chitin finally obtained in atmosphere for 30 days or more and then scaling the appearance according to the grade of poor (yellowish color) or good (white color). In addition, the viscosity was determined by using 0.5% chitosan solution in 1% aqueous acetic acid solution and using a Brookfield Viscometer (#4 spindle, at 60rpm).

Example 1

300g of waste crab shells (Chionoecetes opilio) was stood upon a water bath at 40 to 50°C for 30 minutes and washed with water until the flesh of the crab was completely removed. The shells were dehydrated by using a dehydrator, soaked in an ethanol bath for 1 hour, dehydrated again and dried in the shade for 10 days to provide 200g of dry crab shells (water content 10%).

Said 200g of dried shells was then crushed by using a mill Mot. WRB 90LB/4P (a product of Dietz-Motoren GmbH & Co. KG, Germany) to a range of 0.5 to 3mm. The crushed shell powder was then introduced into a reactor equipped with a

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mechanical stirrer; and thereto 5L of 1N HCl aqueous solution at 0°C was added. The mixture was maintained at 0°C for 20 hours while stirring at the speed of 50rpm, warmed rapidly to 15°C and maintained at that level for 4 hours.

5 The material thus treated was filtered and soaked in water. The pH of the solution containing the material was adjusted to 7-8 by the addition of 5wt% NaOH solution and the solution was stood up for 6 hours. The crude chitin obtained after filtration was washed twice with 3L of acetone and dried in a vacuum oven at 30°C for 36 hours.

10 Subsequently, the dried crude chitin was soaked in 6L of 5% (by weight) NaOH aqueous solution and heated at 90°C for 3 hours while introducing nitrogen gas continuously. After the treatment with the NaOH solution, the pH of the solution containing the crude chitin was adjusted to 7-8 by the addition of 2N HCl solution and the crude chitin was filtered, soaked in 6L of deionized water for 6 hours, filtered, washed twice with 3L of acetone, filtered and dried in a vacuum oven at 30°C for 12 hours.

15 The chitin obtained by the above-mentioned procedures was again subjected to the NaOH treatment process. The chitin was filtered, washed with deionized water five to ten times, soaked in 6L of deionized water for 3 days, filtered and dried under a vacuum to obtain 50g of a biomedical grade chitin.

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Example 2

The procedures described in Example 1 were repeated except that the HCl treatment process was conducted in one
5 step at 0°C for 48 hours.

Example 3

The procedures described in Example 1 were repeated
10 except that the HCl treatment process was conducted in one step at 5°C for 24 hours.

Example 4

15 The procedures described in Example 1 were repeated except that the HCl treatment process was conducted in one step at -8°C for 40 hours.

Examples 5 and 6

20 These examples are intended to show the effect of the condition(e.g. temperature and time) in the HCl treatment on the whiteness level of the chitin produced. The procedures described in Example 2 were repeated except that the HCl
25 treatment process was conducted at 15°C for 8 hours and at 10°C for 24 hours, respectively.

Comparative Example 1

200g of dried crab shells was crushed to a particle size ranging from 0.5 to 3mm. The crushed shell powder was introduced into a reactor equipped with a mechanical stirrer; and thereto 5L of 2N HCl solution was added. The mixture was stirred for 5 hours, cooled to 0°C and stirred again at that temperature for 48 hours. The material so obtained was filtered, washed with water, soaked in 1N NaOH aqueous solution at 100°C for 12 hours, washed with water and dried to provide 80g of chitin.

The experiment conditions employed in the above Examples 1 to 6 and Comparative Example 1 and the properties of the chitin produced are summarized in Table 1.

20

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Table 1

Treatment Conditions and Properties		Example						Comparative Example
HCl Treatment	Conc.	1N	2N	3N	4N	5N	6N	1
	Primary Temp. (°C)	0	0	5	-8	15	10	room Temp.
	Primary Time (hr)	20	48	24	40	8	24	5
	Secondary Temp. (°C)	15						0
	Secondary Time (hr)	4						48
	Conc.	5% weight						1N
NaOH Treatment	Temp. (°C)	90						100
	Time (hr)	3						12
Property	Whiteness	Good	Good	Good	Good	Poor	Poor	Poor
	CaCO ₃ (g)	≤ 0.1						0.89
	Protein (g)	≤ 0.1						≤ 0.5
	Phosphorus (ppm)	≤ 60ppm						≤ 113ppm*

* In Comparative Example 1, other heavy metallic components than K, Ca, Mg, Fe, Ba, Zn (detected in chitin prepared by the present method) were also detected.

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As shown in Table 1, the chitin prepared in accordance with the present invention has a high purity, high molecular weight and good whiteness property.

5 Example 7

10 To a 4L 3-necked flask were added 45g of said chitin obtained in Example 1 and 3L of 40wt% NaOH aqueous solution and the resultant mixture was heated at 90°C for 7 hours while introducing nitrogen gas continuously. The crude chitosan thus obtained was washed with deionized water until the pH of the washes became neutral, filtered, soaked in 3L of deionized water for 2 days, filtered and then immediately soaked in 3L of 10% ethanol aqueous solution for 3 days. The crude
15 chitosan was collected and dried in a vacuum oven at 40°C for 2 days(primary NaOH treatment).

20 To the dried chitosan was added 3L of 40wt% NaOH aqueous solution; and the resultant mixture was heated at 90°C for 3 hours while introducing nitrogen gas continuously. The chitosan thus obtained was subjected to the washing procedures as described in the primary NaOH treatment to obtain a chitosan possessed with the deacetylation degree of 90% or more(secondary NaOH treatment).

25 The chitosan was then subjected to the primary and secondary NaOH treatments again to obtain a chitosan possessed with the deacetylation degree of 92% or more.

Examples 8 to 12

5 These Examples show the effect of the change in the reaction condition on the properties of the chitosan produced. The procedures as described in Example 7 above were repeated except that the reaction conditions in the NaOH treatment were modified as shown in Table 2.

Comparative Example 2

10 To a flask were added 40g of the chitin obtained in Comparative Example 1 above and 2.4L of 40wt% NaOH aqueous solution. The mixture was heated at 115°C for 6 hours, washed, filtered and dried in a vacuum oven to produce
15 chitosan.

20 The experiment conditions employed in the above Examples 7 to 12 and Comparative Example 2 and the properties of the chitosan produced are represented in Table 2.

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Table 2

Table 2											Comparative Example
Example											2
Treatment Conditions and Properties										115°C	
		7	8	9	10	11	12				
NaOH Treatment	Primary Temp (°C)	90	120	reflux	90	120	reflux	6			
	Primary Time (hr)	7	3	3	7	3	3				
	Secondary Temp (°C)	90	120	reflux							
	Secondary Time (hr)	3	3	3							
	Whiteness	Good	Good	Poor	Poor	Poor	Poor	Poor			
	Decacetylation Degree	≥92%	≥92%	≥92%	≥85%	≥85%	≥85%	0.8			
Property	D u r a b i l i t y	Decacetylation Degree	≤ 0.1							≤ 0.3	
		CaCO ₃ (%)	≤ 0.1							≤ 113ppm*	
		Protein (%)	≤ 60ppm								
		Metal (ppm)	≤ 2500								
Viscosity (cps)											

...ative Example 2, other heavy metallic components than K, Ca, Mg, Fe, ... and by the present method) were also detected.

* In Comparative Example 2, other heavy metallic components than K, Ca, Mg, Fe, Ba, Zn (detected in chitin prepared by the present method) were also detected.

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As shown in Table 2, the present invention provides a chitosan possessed with a higher level of deacetylation, purity and molecular weight by way of repeating the NaOH treatment and washing procedures twice or more. Further, as in the above Comparative Example 2, heat treatment at a high temperature results in the chain breakage and poor color of the chitosan. The chitosan obtained in accordance with the present invention is particularly useful for various applications in the medicinal field, which requires chitosan of a high purity.

While the invention has been described in connection with the above specific embodiments, it should be recognized that various modifications and changes as may be apparent to those skilled in the art to which the invention pertains may be made and also fall within the scope of the invention as defined by the claims that follow.

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What is claimed is:

1. A process for preparing a high purity chitin, which comprises the steps of:

(A) drying crustaceous shells and crushing the dried

shells to provide a finely crushed powder;

(B) digesting initially the powder in a 0.4 to 3M HCl solution at a temperature ranging from -10 to 10°C for a

period ranging from 10 to 25 hours and, thereafter, further digesting the powder in the HCl solution at a temperature ranging from 10 to 20°C for a period ranging from 2 to 8 hours;

(C) washing with water, filtering, rinsing with an organic solvent and drying the HCl-treated material to provide a crude chitin;

(D) soaking and heating the crude chitin in a 2 to 10 wt% NaOH solution; and

(E) washing with water, filtering, rinsing with the organic solvent and drying the NaOH-treated chitin to obtain said high purity chitin.

2. The process of claim 1 wherein said initial digestion in step(B) is carried out at a temperature ranging from -5 to 5°C for a period ranging from 15 to 20 hours and the further digestion therein is carried out at a temperature ranging from 12 to 18°C for a period ranging from 3 to 5 hours.

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3. The process of claim 1 wherein the drying of the shells in step(A) is conducted in the shade until the water content of the shells becomes 12% or less.

5 4. The process of claim 1 wherein the digestion in step(B) is conducted with stirring by means of a mechanical stirrer at a speed ranging from 50 to 200rpm.

10 5. The process of claim 1, wherein the digestion in step(B) is carried out in one step at a temperature ranging from -10 to 8°C for a period ranging from 20 to 60 hours.

15 6. The process of claim 5 wherein said one-step digestion is carried out at a temperature ranging from -5 to 5°C for a period ranging from 30 to 50 hours.

20 7. The process of claim 1 wherein the solvent employed in steps(C) and (E) is selected from the group consisting of ethanol, methanol, acetone, tetrahydrofuran, dioxane and methyl ethyl ketone.

8. The process of claim 1 wherein the soaking with the NaOH solution in step(D) is carried in the presence of an inert gas.

25 9. The process of claim 1 wherein steps(D) and (E) are

repeated twice or more.

10. The process of claim 9 wherein the high purity chitin produced is soaked in deionized water for 3 to 4 days, filtered and dried.

11. A process for preparing a biomedical grade chitosan from the chitin prepared in accordance with the process of claim 1 or 5, which comprises the steps of:

(a) soaking the chitin in a NaOH solution at a temperature ranging from 80 to 100°C for a period ranging from 2 to 12 hours to provide a crude chitosan;

(b) filtering, washing with water, soaking in deionized water and then in an aqueous mixture of a water-miscible organic solvent, filtering and drying the crude chitosan; and

(c) repeating steps (a) and (b) by using the crude chitosan once or more to provide said biomedical grade chitosan.

12. The process of claim 11 wherein the aqueous mixture comprises 5 to 30% by weight of the solvent.

13. The process of claim 12 wherein the solvent is selected from the group consisting of acetone, ethanol, methanol, isopropanol, dioxane, tetrahydrofuran and methyl ethyl ketone.

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14. The process of claim 11 wherein step(a) is carried out in the presence of an inert gas.

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Abstract

5 The present invention provides a process for preparing a biomedical grade chitin of a high purity and whiteness with a high molecular weight and a biomedical grade chitosan of a high degree of deacetylation and high molecular weight from the chitin produced in accordance with the present invention.

Specifically, the process for preparation of the chitin in accordance with the present invention comprises the steps of:

10 of: (A) drying crustaceous shells and crushing the dried shells to provide a finely crushed powder;

(B) digesting initially the powder in a HCl solution at a temperature ranging from -10 to 10°C for a period ranging from 10 to 25 hours and, thereafter, further digesting the powder in the HCl solution at a temperature ranging from 10 to 20°C for a period ranging from 2 to 8 hours;

15 (C) washing with water, filtering, rinsing with an organic solvent and drying the HCl-treated material to provide a crude chitin;

20 (D) soaking and heating the crude chitin in a NaOH solution; and

(E) washing with water, filtering, rinsing with the organic solvent and drying the NaOH-treated chitin to obtain the high purity chitin.

25